

(see identification of Rho antagonist section).

SEQUENCE of (known) Rho antagonist C3 used in the experiments

Nucleotide sequence including part of the plasmid GST sequence. The vector with the GST sequence is commercially available and thus the entire GST sequence including the start was not sequenced. It was desired to determine only the sequence 3' to the thrombin cleavage site which releases C3 from the GST sequence. The thrombin cleavage site is shown with an arrow and is located just to the left of the underlined nucleotide sequence below (i.e. the arrow shows the thrombin cleavage site). The underlined sequence shows additional coding sequence translated in our recombinant protein that is not reported in the literature.

Both strands were sequenced to verify that there were no errors in the sequence.

↓

5' GTG GCG ACC CTT CCC AAA TCG GAT CTG GTT CCG CGT GGA TCC TCT AGA
GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC
CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT
GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA
CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA
TTT AGA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA
AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA
CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT
AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC
GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
GCT ATC AAT CCT AAA TAA 3'

Nucleotide sequence of recombinant C3 protein: the sequence given below represents the entire coding sequence for the Rho antagonist used in the experiments mentioned herein. It is similar to the sequence shown above but does not include the GST portion which when the protein is made is enzymatically removed with thrombin.

1 GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA
51 AAAGGCTTAT TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA
101 AAGCTTGGGG TAATGCTCAG TATAAAAAGT ATGGACTAAG CAAATCAGAA
151 AAAGAAGCTA TAGTATCATA TACTAAAAGC GCTAGTGAAA TAAATGGAAA
201 GCTAAGACAA AATAAGGGAG TTATCAATGG ATTCCTTCA AATTTAATAA
251 AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA
301 AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT

351 TCAAAACACT CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG
 401 AAAAGGCTAA AGCTAAGTTT TTAAATAAAG ATAGACTTGA ATATGGATAT
 451 ATTAGTACTT CATTAATGAA TGTTTCTCAA TTTGCAGGAA GACCAATTAT
 501 TACAAAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT ATTGACCCTA
 551 TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT
 601 TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT
 651 TACAGCAACA ATGATGGGCA CAGCTATCAA TCCTAAATAA

Amino acid sequence (one letter code)

Translation of the above sequence to show amino acid sequence. Amino acids in bold, highlight differences from published sequence (Popoff et al. (1990) Nucl. Acid. Res. 18:1291. EMBL accession no. X511464.) The 11 N-terminal sequences are additional; there is a single amino acid change of an alanine (hydrophobic) to glutamic acid (Q).

GSSRVDLQAC NAYSINQKAY SNTYQEFTNI DQAKAWGNAQ YKKYGLSKSE
 KEAIVSYTKS ASEINGKLRQ NKGVINGFPS NLIQVELLD KSFNKMKTPE NIMLFXGDDP
 AYLGTEFQNT LLNSNGTINK TAFEKAKAKF LNXDRLEYGY ISTSLMNVSQ FAGRPITKF
 KVAKGSKAGY IDPISAF**Q**GQ LEMLLPRHST YHIDDMRLSS DGKQIIITAT MMGTAINPK

Number of amino acids: 229

Molecular weight: 25507.5

Theoretical pI: 9.43

Amino acid composition:

Ala (A)	18	7.9%
Arg (R)	6	2.6%
Asn (N)	18	7.9%
Asp (D)	10	4.4%
Cys (C)	1	0.4%
Gln (Q)	12	5.2%
Glu (E)	10	4.4%
Gly (G)	16	7.0%
His (H)	2	0.9%
Ile (I)	18	7.9%

Leu (L)	17	7.4%
Lys (K)	23	10.0%
Met (M)	7	3.1%
Phe (F)	10	4.4%
Pro (P)	7	3.1%
Ser (S)	20	8.7%
Thr (T)	14	6.1%
Trp (W)	1	0.4%
Tyr (Y)	11	4.8%
Val (V)	6	2.6%

Asx (B)	0	0.0%
Glx (Z)	0	0.0%
Xaa (X)	2	0.9%

Total number of negatively charged residues (Asp + Glu): 20

Total number of positively charged residues (Arg + Lys): 29

Estimated half-life:

The N-terminal of the sequence considered is G (Gly).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).

>20 hours (yeast, in vivo).

>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 26.88

This classifies the protein as stable.

Aliphatic index: 75.07

Grand average of hydropathicity (GRAVY): -0.479]

Please also delete the following passage found on pages 46 to 51;

[SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: LISA MCKERRACHER

(ii) TITLE OF INVENTION: Methods for making and delivering Rho-antagonist tissue adhesive formulations to the injured mammalian central and peripheral nervous systems and uses thereof

(iii) NUMBER OF SEQUENCES: 3

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: BROULLETTE KOSIE

(B) STREET: 1100 RENE-LESVEQUE BLVD WEST

(C) PROV/STATE: QUEBEC

(D) COUNTRY: CANADA

(E) POSTAL/ZIP CODE: H3B 5C9

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: ASCII (TEXT)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) ATTORNEY/AGENT INFORMATION:

(A) NAME: RONALD S. KOSIE

(B) REGISTRATION NO.: 28,814

(C) REFERENCE/DOCKET NO.: 06447-003-US-2

(D) TEL. NO.: (514) 397 8500

(E) FAX NO.: (514) 397 8515

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:

(B) TYPE:

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTG GCG ACC CTT CCC AAA TCG GAT CTG GTT CCG CGT GGA TCC TCT AGA

5 10 15
 GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT
 20 25 30
 TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG
 35 40 45
 GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA
 50 55 60
 GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA
 65 70 75 80
 AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA
 85 90 95
 CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA
 100 105 110
 AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA
 115 120 125
 TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT
 130 135 140
 TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT AGA CTT GAA TAT
 145 150 155 160
 GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA
 165 170 175
 CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT
 180 185 190
 ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT
 195 200 205
 AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT
 210 215 220
 AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT
 225 230 235 240
 AAA TAA

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:

(B) TYPE:

(C) STRANDEDNESS:

(D) TOPOLOGY:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA 50
AAAGGCTTAT TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA 100
AAGCTTGGGG TAATGCTCAG TATAAAAAGT ATGGACTAAG CAAATCAGAA 150
AAAGAAGCTA TAGTATCATA TACTAAAAGC GCTAGTGAAA TAAATGGAAA 200
GCTAAGACAA AATAAGGGAG TTATCAATGG ATTCCTTCA AATTTAATAA 250
AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAATGAA GACCCCTGAA 300
AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT 350
TCAAAACACT CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG 400
AAAAGGCTAA AGCTAAGTTT TTAAATAAAG ATAGACTTGA ATATGGATAT 450
ATTAGTACTT CATTAATGAA TGTTTCTCAA TTTGCAGGAA GACCAATTAT 500
TACAAAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT ATTGACCCTA 550
TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT 600
TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT 650
TACAGCAACA ATGATGGGCA CAGCTATCAA TCCTAAATAA

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:

(B) TYPE:

(C) STRANDEDNESS:

(D) TOPOLOGY:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(ix) FEATURE:

(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GSSRVDLQAC NAYSINQKAY SNTYQEFTNI DQAKAWGNAQ YKKYGLSKSE 50
KEAIVSYTKS ASEINGKLRQ NKGVINGFPS NLIKQVELLD KSFNKMKTPE 100